IDENTIFICATION OF RAPD MARKERS FOR THE DETECTION OF THE YELLOW RUST RESISTANCE GENE YR_{15} IN WHEAT .

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Received on: 25/6/2005 *Accepted on: 6 /8 /2005*

ABSTRACT

The objective of this study was to identify RAPD markers linked to the yellow rust resistance gene, Yr_{15}, in F_2 population of wheat by using the bulked segregant analysis (BSA) procedures with RAPD markers. F_2 populations had been derived from the cross between Gemmiza-3 and the monogenic line which contained the yellow rust gene, Yr_{15}, while, Gemmiza-3 is susceptible to that disease. Bulk segregant analysis was used in conjunction with RAPD analysis. After Mapmaker linkage analysis on the F_2 population, the two closest markers primers (OPB13 and UBC321) were shown to be linked to Yr_{15} within a distance of about 20.9 cM . A standard maximum-likelihood technique was employed to analyze the linkage between Yr_{15} and two linked marker loci. The map distances between Yr_{15} gene and OPB13 and UBC321 primers were 7.7 and 13.2 cM with LOD scores of 11.6 and 7.9, respectively. Therefore, the order OPB13, Yr_{15} and UBC321 primer gave the highest value of the log-likelihood function. Thus, the mapping efforts allowed finding two molecular markers flanking the Yr_{15} gene.

In conclusion, the present study indicated that RAPD markers, combined with bulk segregant analysis, could be used to identify molecular markers linked to yellow rust resistance gene in wheat. Once these markers are identified, they can be used in wheat breeding as a selection tool in early generation.

Key words *: RAPD Markers, Wheat , Yr_{15}, Yellow Rust Resistance .*

INTRODUCTION

The main target of the agricultural policy, in Egypt, is to increase the wheat production, in specific, as well as the other food crops in general, to decrease the gap between wheat production (50-55%) and the annual requirements (about 12 million tons). In Egypt, wheat rust diseases are still the main factors for eliminating and decreasing the longevity of the Egyptian wheat cultivars.

During the last three decades, yellow rust disease, caused by the fungal pathogen (*Puccinia striiformis*) was very destructive in different seasons and hit most of the Egyptian wheat cultivars; i.e., in 1967, 1995, 1997 and slight epidemic that occurred in 1985 and 1998 (El-Daoudi *et al*, 1980 and 1996).

Bulked segregant analysis (BSA) is a method to identify molecular markers linked to a gene of interest without having to construct a map of the genome (Michelmore *et al*, 1991). BSA has been successfully used to develop RAPD markers for wheat resistance to yellow rust (Chen *et al*, 1998). Different DNA markers linked to the gene, Yr_{17} in bread wheat had been reported (Robert *et al*, 2000). More recently, William *et al*, (2003) as Yr_{29} .

The search for molecular markers for disease resistance has become a high profile activity in many laboratories. This has two main bases; firstly, the hope that a completely linked marker would enable the cloning of the tagged resistance gene; and secondly, the potential of markers to aid the selection (or enrichment) of genotypes with desired resistance genes often without the need of disease testing. In the latter respect, closely linked markers would be valuable for assembling resistance gene combination, strategy often to enhance the durability of resistance based on major genes (McIntosh *et al*, 2001). The present paper describes the use of RAPD method for the tagging and mapping of the important yellow rust resistance gene (Yr_{15}) with RAPD markers, using the F_2 population.

MATERIALS AND METHODS

Plant material :

Identification of RAPD markers linked to Yr_{15} gene were carried out on segregating F_2 population, derived from a cross between the resistant monogenic line, Yr_{15} , and the susceptible cultivar, Gemmiza-3. The cross was made during the season of 2001 and was selfed in 2002 to produce the F_2 population.

Yellow rust resistance evaluation :

For evaluating against yellow rust disease, in the 2003 season, the F_2 population was grown under field conditions at the Experimental Farm of Field Crop Research Institute, Sakha Agricultural Research Station, Egypt. At the late tillering and beginning of booting stages, disease tests were carried by using a mixture of the common races under the Egyptian conditions. After twenty days from inoculation, green leaves from young F_2 plants were collected for RAPD analysis.

DNA extraction :

Genomic DNA was extracted from fresh leaves of monogenic lines, Gemmiza-1 and individual F_2 plants, using CTAB (Saghai - Maroof *et al*, 1984). RNA was removed from the DNA preparation by adding 10µl of RNAase (10mg /ml) and, then, incubated for 30 min at 37°C. Sample DNA concentration was quantified by using a spectrophotometer (Beckman Du-65).

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PCR amplification :

Six primers (Table 1), previously tested by Sun *et al.* (1997), were used in this investigation to amplify the templated DNA. Amplification reaction volumes were 25 µl , each containing 1 x PCR buffer with $MgCl_2$ (50 mM KCl, 10 mM Tris-HCl (pH = 9.0), 2 mM $MgCl_2$ and 1% trition x-100), 200 µM each of dATP, dCTP, dGTP and dTTP, 50 PM primer, 50 ng template DNA and 1.5 µ of tag polymerase. Reaction mixtures were overlaid with 15 µl mineral oil and exposed to the following conditions: 94°C for 3 min, followed by 45 cycles of 1 min. at 94°C, 1 min. at 36°C, 2 min. at 72°C, and a final 7 min. extension at 72°C.

Amplification products were visualized with DNA marker on 1.6% agarose gel with 1x TBE buffer and detected by staining with an ethidium bromide solution for 30 min. Gels were, then, destained in deionized water for 10 min. and photographed on Polaroid films under UV light.

Table 1: Primer sequences used in the detection of Yr_{15}

Name of primer	M.W	Primers sequence (5' to 3')
UBC475	3003	CCAGCGTATT
UBC532	3092	TTGAGACAGG
UBC321	3052	ATCTAGGGAC
OPB8	3013	GTCCACACGG
OPB13	2915	TTCCCCCGCT
OPF15	2948	CCAGTACTCC

Bulked segregant analysis of the yellow rust resistance genes :

Four different bulks were created for both phenotypic classes of plants, as follows: bulks 1 R (resistant) and 1S (susceptible), respectively, a mix of equal amounts of DNA from five resistant and five susceptible was chosen at random.Each of the RAPD primers were simultaneously screened on these four DNA bulks and on the parental cultivars monogenic cultivar and Gemmiza -3. Based on the e
DNA bulks, 39 individual F_2 plants were analy
with cosegregating primers to confirm RAPD marker linkage to the Yr_{15} gene .

Data analysis :

Goodness of fit to a 3:1 ratio was calculated for RAPD marker by Chi-square test. A regression analysis was performed between the RAPD marker and the values of yellow rust resistance gene of the F_2 lines (Moreno and Gonzales, 1992).

Linkage analysis :

Map manager QTX Version 0.22 (Meer *et al.* 2001) was used to analyze the linkage relationship of RAPD markers detected from bulked segregant analysis. Linkage was declared when a log of the likelihood ratio (LOD) threshold of 3.0 and maximum distance was 50 cM. The Kosambi's mapping function was used.

RESULTS AND DISCUSSION

Identification of RAPD markers linked to Yr_{15} :

In an attempt to facilitate the pyramiding of adult plant resistance genes in wheat, using molecular markers, it was necessary to identify the markers, which were linked to the yellow rust resistance genes and were simple and accurate to be used. For this purpose, the cross between the resistant Yr_{15} donor line (monogenic line) and Gemmiza-3 was made, as the source of the segregating population to identify the RAPD markers linked to the yellow rust resistance gene. The monogenic line possessed the Yr_{15} gene, while Gemmiza-3 was susceptible under artificial infection. In the F_2 population, bulked DNA from the individuals, differing in resistance to yellow rust, used as a template for amplification with each arbitrary 10-mer oligonucleotide primers. A total of six primers (Table 2) previously tested by Sun *et al.* (1997), for screening Yr_{15}, were used to identify Yr_{15} in the donor parent and F_2 population. Primers that gave clear, distinguishable and reproducible patterns were considered for analysis.

The 700bp fragment, amplified by UBC321, was present in the monogenic line (resistant parent) and was absent in the susceptible parent, Gemmiza-3 .This marker (UBC321), also, was present in the resistant bulked DNA, but not in the susceptible bulked DNA (Fig. 1).

The 800bp fragment, amplified by OPB13, was present in the monogenic line (resistant parent) and was absent in the susceptible parent, Gemmiza-3 This marker (OPB13), also, was present in the resistant bulked DNA, but not in the susceptible bulked DNA (Fig. 2).

The two markers, UBC321 and OPB13 primers, were further used to check its linkage to the yellow rust resistance gene (Yr_{15}) by using the segregating F_2 population, derived from the cross between the resistant Yr_{15} donor line (monogenic line) and Gemmiza-3.

Table 2: Number of amplification and polymorphic products, using six primers, in wheat cultivars and their somaclones.

Primer name	Nucleotide sequence (5`- 3`)	No. of amplification products a	No. of polymorphic products b	Polymorphism b/a (%)
UBC475	CCAGCGTATTTTGA	7	7	100%
UBC532	GACAG	2	2	100%
UBC321	ATCTAGGGAC	14	11	79%
OPB8	GTCCACACG	11	10	91%
OPB13	TTCCCCCGCT	6	6	100%
OPF15	CCAGTACTCC	5	5	100%

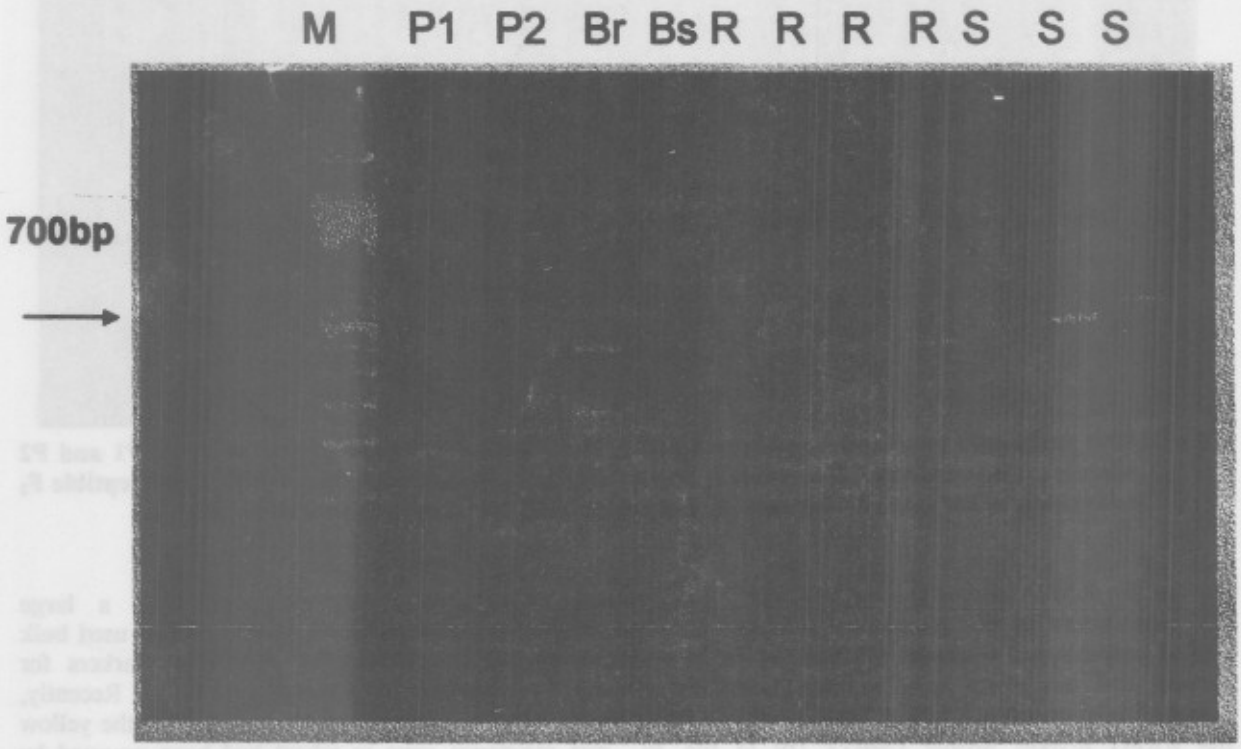

Fig 1 : RAPD fragments produced by primer UBC321, M: Molecular weight , followed by P1 and P2 parents , Monogenic and Gemmiza-3, respectively., Br, bulk resistance; Bs bulk susceptible F_2 individuals in the cross, Monogenic X Gemmiza-3 ((R: resistant ; S: susceptible).

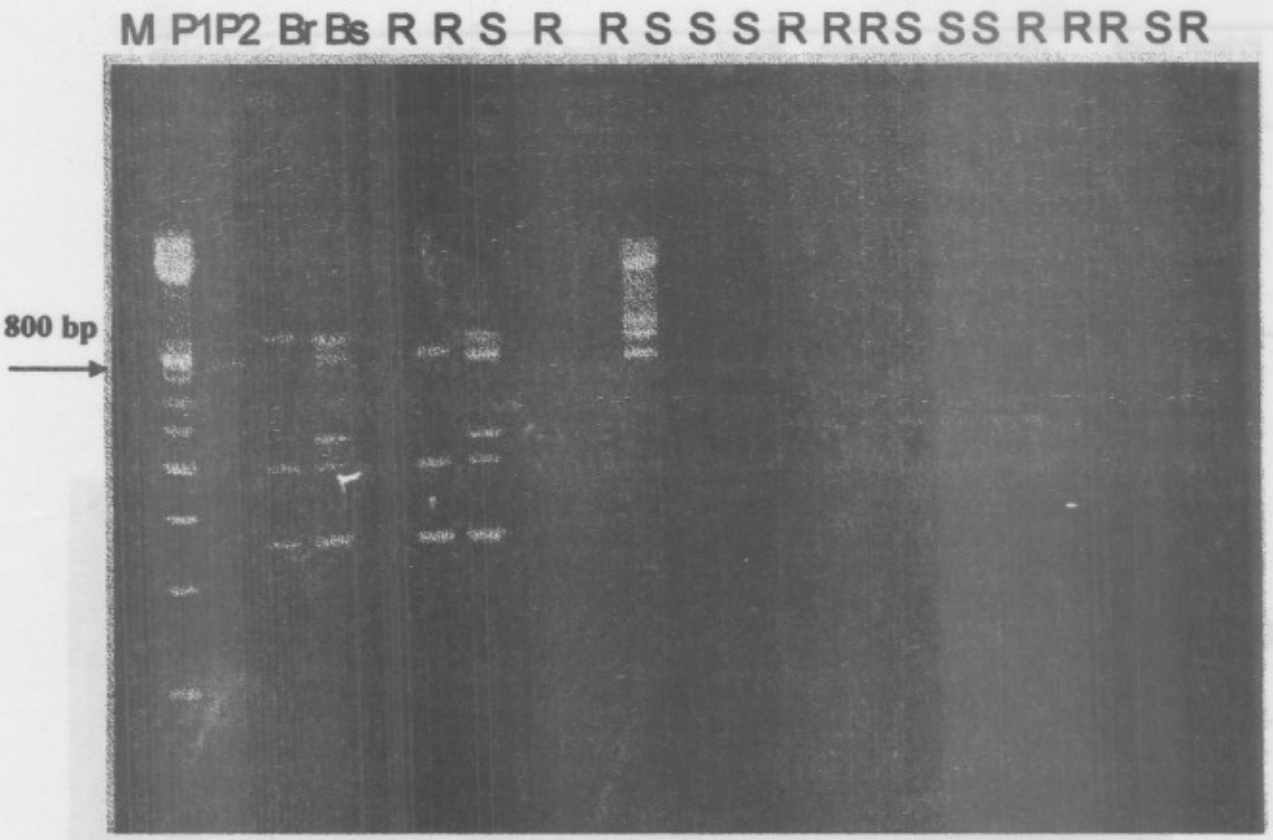

Fig 2 : RAPD fragments produced by primer UPB13, M: Molecular weight , followed by P1 and P2 parents , Monogenic and Gemmiza-3, respectively., Br, bulk resistance; Bs bulk susceptible F_2 individuals in the cross Monogenic X Gemmiza-3 ((R: resistant ; S: susceptible).

For the RAPD marker primer UBC321, 23 of the 39 individuals in the population exhibited the amplified polymorphic fragment (700bp), while, the remaining did not (Fig. 1). The ratio fitted the expected Mendelian ratio, 3:1 (X^2= 2.032, p< 0.01). In the RAPD marker primer OPB13, 29 of the 39 individuals, in the population, exhibited the amplified polymorphic fragment (800bp), while, the remaining did not (Fig. 2). The ratio fitted the expected Mendelian ratio 3:1 (X^2= 0.0085, p< 0.01).

A regression analysis was performed to test the significance of the linkage between Yr_{15} and the polymorphic markers. The results showed that the regression analysis for the OPB13 and UBC321 primers were significant. The calculated r^2 for OPB13 and UBC321 were 0.645 and 0.362, respectively. This indicates that the two markers were linked with the yellow rust resistant gene, Yr_{15}. Barakat *et al,* (2001) identified one RAPD marker (primer 7_{700}) linked to the leaf rust resistance gene, Lr_{29} , in F_2 wheat population. It allowed, then, to explain 27.5% of the total phenotypic variation. Using bulk segregates, in conjunction with RAPD analysis, polymorphic fragment was found to be perfectly linked to one of the wheat yellow rust resistance gene in a large segregating population, Chen *et al* (1998) used bulk segregant analysis to develop RAPD markers for wheat resistance to yellow rust. Recently, identification of RAPD markers linked to the yellow rust resistance gene in wheat had been reported by Motawei *et al* (2003).

Mapmanager analysis :

After mapmaker linkage analysis on the F_2 population, the two closest markers (OPB13 and primer UBC321) were shown to be linked to Yr_{15} within a distance of about 20.9 cM (Fig. 3). A standard maximum-likelihood technique was employed to analyze the linkage between Yr_{15} and the two linked marker loci. The map distances between Yr_{15} gene and OPB13 and UBC321 primers were 7.7 cM and 13.2 cM with LOD scores of 11.6 and 7.9, respectively. Therefore, the OPB13, Yr_{15} and primer UBC321 order gave the highest value of the log-likelihood function. Thus, the mapping efforts allowed finding two molecular markers flanking the Yr_{15} gene. The Yr_{15} gene was identified in 1B homologous group of wheat genome by McIntosh (1988). Similar results were

reported for identifying molecular markers for linkage with Yr_{15} gene. A 2.8-kb fragment, produced by the *Nor* RFLP probe and 1420-bp PCR product generated by the RAPD OPB13 primer showed linkage, in coupling, with the Yr_{15} gene (Sun *et al*, 1997). The map distances between OPB13-Yr_{15}-Nor were 27.1 cM and 11.0 cM for the first and second intervals, respectively. Also, Chague *et al*, (1999) found that the yellow rust resistance gene, Yr_{15} was surrounded by flanking PCR markers, UBC199700 and GWE33, at about 5 cM from each side. The development of an RFLP map had been of much difficult in wheat than in most other crops due to the polyploid nature of the crop, a high proportion of repetitive DNA, and, unusually, low levels of polymorphism within the genome (Chao *et al*., 1989). Levels of polymorphism in wheat, detected by RAPD analysis, were similar to those detected by RFLP analysis (Devos *et al* ,1992).

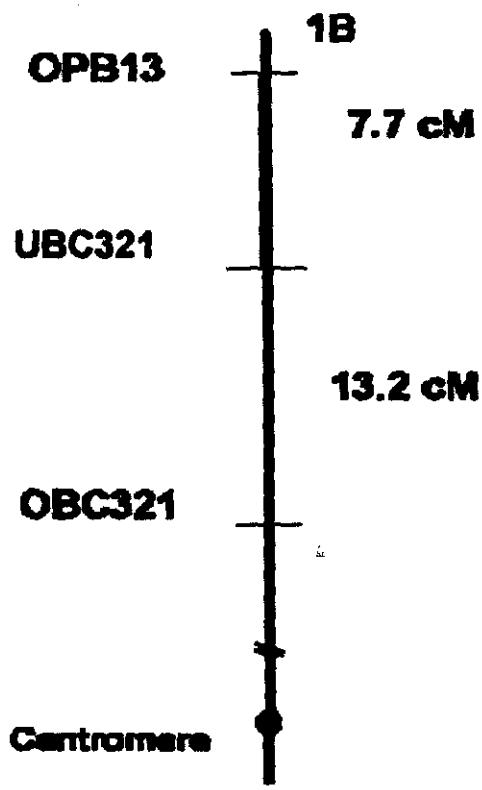

Fig. 3: Linkage map showing the region near the Yr_{15} gene. All distances are given in centiMorgan using Kosambi's mapping function.

The present study indicated that RAPD markers, combined with bulk segregant analysis, could be used to identify molecular markers linked to yellow rust resistance gene in wheat. Once these markers are identified, they can be used in breeding programs, as a selection tool in early generations.

ACKNOWLEDGEMENTS

This research project was carried out under the Program of the National Strategy for Biotechnology and Genetic Engineering. The program is administrated by the Science and Technology Center at the Academy of Scientific Research and Technology in Egypt.

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الملخص العربي

تحديد جين Yr_{15} المقاوم للصدأ الأصفر في القمح باستخدام طريقة RAPD.

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كان هدف هذه الدراسة هو التعرف على دلائل جزيئية باستعمال تكنيك ال RAPD و المرتبطة بجين (Yr_{15}) المقاوم للصدأ الأصفر فى عشيرة من الجيل الثانى فى القمح و الناتجة من تهجين صنف القمح "جميزه -٣" القابل للإصابة بالصدأ الأصفر و سلالة "ال Monogenic" المحتوية على جين المقاومة للصدأ الأصفر (Yr_{15}) . وقد تم اجراء العدوى الصناعية داخل الصوبة فى محطة بحوث سخا و باستخدام تحليل bulk segregant analysis المرتبطة مع تحليل ال RAPD. وأمكن تلخيص أهم النتائج كما يلي :-

- وجد ان primer OPB13 و ال primer UBC321 مرتبطان بجين المقاومة Yr_{15} و بمسافة قدرها ٢٠,٩ سنتيمورجان.
- بواسطة التحليل وجد أن المسافة على الخريطة ما بين الجين Yr_{15} و ال primer OPB13 كانت ٧,٧ سنتيمورجان ، بينما كانت المسافة بين الـ primer UBC321 ١٣,٢ سنتيمورجان .